Remarks

Rejection of Claims 1-7, 14, 15, 17-24, and 41-44 under 35 U.S.C. § 103(a)

Claims 1-7, 14, 15, 17-24, and 41-44 stand rejected as unpatentable over Zhang et al., U.S. 6,972,013, Glasspool-Malone (Mol. Therapy, 2:140-146, 2000), Bureau et al., U.S. 6528315, in view of Ruben (US 2003/0186904), Arbeit (US 6838430), Blott (US 2007/0141128), and Miller (US 4846181). Zhang, Glasspool-Malone, and Bureau are designated as the primary references. This rejection is respectfully traversed.

The Office Action alleges that "the instant invention would have been obvious to one of ordinary skill in the art because the administration and expression of **nucleic acids encoding wound healing polypeptides** at the site of the wound and using electroporation for enhancing nucleic acid delivery to cells in the wound area were well known to accelerate wound healing, as taught previously by many including Zhang, Reuben, Blott, and Arbeit. Furthermore, it would have been obvious to enhance nucleic acid delivery to target cells, including to cells and tissues at wound sites, using electrocurrent because **electroporation** of cells in vitro and in vivo was well known in the art for enhancing nucleic acid delivery as taught previously by Zhang, Glasspool-Malone, Bureau and Ruben. And Miller taught the use of applying electric current to wounds to stimulate wound healing more than twelve years before the filing of the instant application." Office Action at paragraph spanning pages 5 and 6.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. §2143. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The present rejection fails in the second and third criteria.

The U.S. Patent and Trademark Office has failed to make a *prima facie* case of obviousness because the prior art references, alone or in combination, fail to teach or suggest all of the limitations of the claims. The second step of the method of claim 1 (and claim 22, the only other independent claim of the rejected claim set) recites "applying an electric field intradermally to the wounded tissue...." Not one of the cited references teaches this step.

- Zhang teaches the **surface** application of an electric filed to **normal** tissue.[1] Zhang also teaches using electrodes having a **needle** configuration, but does not teach their use at wound tissue.[2]
- Glasspool-Malone teaches the intradermal application of an electric field to **normal** skin.[3]
- Miller teaches the **surface** application of an electric filed to **wound** tissue.[4]
- Bureau teaches **non-invasive** electroporation of **normal** tissue using electrodes placed in contact with the skin.[5]
- Ruben teaches use of electroporation in **tissue culture** only.[6]
- Blott teaches applying nucleic acid vectors to wounds, but nothing at all about applying an electric field.
- Arbeit teaches transfection with nucleic acid but does not teach anything about electric field application.

Thus, not a single reference teaches the second step of the method, i.e., applying an electric field intradermally to a wounded tissue. The U.S. Patent and Trademark Office obscures this fact by cobbling together a large number of references which each teach an isolated element of the claimed method. But not a single reference teaches one of the two method steps.

Given the failure of the cited prior art to teach intradermal administration of an electric field to wound tissue, one must ask whether there would have been any reasonable expectation of success if one were motivated to try this. Based on the large differences between normal skin and wound tissue, one of skill in the art would not have had a reasonable expectation.

Wound tissue is very different in its properties from normal skin. Lokmic (*Wound Rep. Reg.* 2006, 14: 277-288; already of record) meticulously documents and characterizes many differences between normal skin and wound tissue. Lokmic used histochemical localization of hypoxia to

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[1] Column 3, lines 55-56 ("applying at least two non-invasive electrodes to the skin tissue site"); column 4, lines 45-46 (electropulsing a thin fold of skin using a caliper electrode")

[2] Column 9, lines 3-4 ("at least one of the electrodes having a needle configuration for penetrating the tissue")

[3] Page 141, paragraph spanning columns 1 and 2 ("A pin electrode consisting of two rows of seven, 7-mm pins (1_5.5-mm gaps) was used to transfer the electric field to the injection site. ")

[4] Column 4, lines 34-35 ("such application can be effected by direct pad application, as indicated in FIG. 1, or can be effected by hydrotherapy, as indicated in Fig. 2, with the pulses being delivered to the wound through active electrode 17 immersed in a saline solution 19.")

[5] Column 4, lines 8-11

[6] Page 69, [0609-0610] ("Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection calcium phosphate mediated transfection, or viral infection....the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell.")

characterize one such difference. Using Hypoxyprobe-1™ which is a substituted 2-nitroimidzaole which binds only to cells that have oxygen concentration less than 14 μM, Lokmic detected hypoxic cells and regions of hypoxia. As shown in Figure 2, normal skin did not label with the probe, whereas wounded tissue displayed changing regions of labeling as the wound healed from 3 days to 3 weeks. Labeled regions in the wound tissue included granulation tissue, and blood vessels, fibrin clot, new epidermis, hair follicles, myofibroblasts, and macrophages.

Lokmic also characterized the differences in the vascular volume of normal skin versus wound granulation tissue. Figure 3 shows that at the peak (7 days) the wound tissue had about 16 % vascularity while the normal skin had only about 3 %.

Lokmic also characterized the differences in the proliferation of normal cells and wounded tissue, using a Ki67 antibody. The difference between day 0 (normal cells) and day 3 (wound tissue) is pronounced, and is quantitated in Figure 4C. At day 3 there are about 125 cells per square meter and at day 0 less than 5 cells per square meter.

Lokmic further characterized the differences between normal skin cells and wounded tissue by measuring apoptosis using a TUNEL label (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling). As shown in Figure 5, the number of apoptotic cells in wound granulation tissue at 2-6 weeks was markedly greater than in normal skin cells as shown at day 0 (Fig. 5C) and the negative control (Fig. 5A). At 3 weeks about 12 apoptotic cells per square meter were observed. At day 0 less than 1 cell per square meter was observed.

Lokmic also characterized the difference in vascular endothelial growth factor-A (VEGF-A) expression using immunohistochemical localization. Again, as seen in Figure 6, the difference between normal skin and the wounded skin is clear. Similarly, vascular endothelial growth factor receptor-2 (VEGFR-2) labeling indicated differences in expression between the two types of tissue as shown in Figure 7. Labeling for VEGF-A was found in wound tissue, vascular sprouts, myofibroblasts, smooth muscle cells, endothelium of arterioles, epidermal cells, hair follicles, and connective tissue. VEGFR2 was observed in wound tissue in vascular sprouts, macrophages, and smooth muscle cells. VEGFR2 is a receptor involved in angiogenesis. See page 284.

Differences in labeling of the two tissue types were also seen when sections were labeled for alpha-smooth muscle actin (α-SMA) (Figure 8) and transforming growth factor-beta (TGF-β) (Figure 9). TGF-β regulates cell proliferation, differentiation, adhesion, migration, Extracellular Matrix (ECM) production, and vascular remodeling. See page 284. Thus Lokmic shows that normal skin and wounded skin are different by a number of important biological and biochemical criteria including connective tissue cell proliferation, apoptosis, hypoxia, marker expression, vascular volume, and remodeling. These confirm our common knowledge and common sense experiences of the differences between normal skin

and wound tissue. See Declaration Under Rule 132, paragraphs 5-6.

Because of the very large and important differences between wound tissue and normal skin, one of ordinary skill in the art would not have had a reasonable expectation that a process that may have been successful in normal skin would be successful in wound tissue.

Moreover, the extent of effect of the combination of the first and second steps on wound healing could not have been predicted. "A greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). Evidence of a greater than expected result may also be shown by demonstrating an effect which is greater than the sum of each of the effects taken separately (*i.e.*, demonstrating "synergism"). *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989).

Indeed, the combination of intradermal electric pulses to wound tissue and delivery of nucleic acids encoding growth factors provides greater than additive results. As shown in Lee et al., ("Electroporatic delivery of TGF-β1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice," *J. Invest. Dermatol.* 123: 791-198, 2004; already of record) the effect of this combination on wound healing is far greater than additive. The effect of the combination on the percentage of actively proliferated epidermal cells at region 2 of the wound edge of the epithelium at day 3 post-wounding is dramatic. Intradermal electric pulses alone yielded about a 20 % yield of actively proliferated epidermal cells, intradermal growth factor-encoding plasmid alone yielded about a 32 % yield, and the combination yielded about a 70 % yield. See Figure 4B. This represents 34 % more than a merely additive result.

Similarly the effect of the combination of steps 1 and 2 of the claimed method on angiogenesis in the granulation tissue at the center of the wound bed at day 7 post-wounding is far greater than additive. The endothelial cell count with intradermal electric pulses alone is undetectable, and with intradermal administration of growth factor-encoding plasmid alone it is about 2-3 cells per field. In contrast, the combination treatment yielded about 24 endothelial cells per field. See Figure 7. Again, by this criterion, the combination yields an effect that is far greater than additive, *i.e.*, eight to twelve-fold greater. Lee states that "indeed the electric effect and gene effect work synergistic[ally] in the genetically diabetic model." Abstract, last line. Lee et al.'s conclusion is reinforced by Sattinger and Goldsmith in their column "Clinical Snippets, " *J. Invest. Dermatol.* 123: vi, 2004 (already of record): "The combination of electric pulses and delivery of TGF-β1 plasmid provided an innovative synergism to treat diabetic wound healing in the db/db (diabetic) mouse. The findings by Lee and co-workers may have a significant

implication for clinical applications."[7] See Declaration Under Rule 132, paragraphs 2-4.

Thus the combination of references would not have rendered the invention obvious to one of skill in the art at the time of the invention because (a) no prior art reference taught intradermal administration of an electric field to wound tissue, and (b) there are so many differences between wounded tissue and normal skin that one of ordinary skill in the art would not have had a reasonable expectation of success that the intradermal electric field administration to normal skin of Glasspool-Malone would be successful when applied to wound tissue. Similarly, one of ordinary skill in the art would not have had a reasonable expectation of success that the surface application of an electric field as taught by Miller would be successful when applied intradermally to wound tissue. Moreover, even if, *arguendo,* a *prima facie* case remains, the very positive and synergistic effect of the treatment on wound healing was unexpected and significant and rebuts the *prima facie* case of obviousness.

Withdrawal of this rejection is therefore requested in view of the record evidence.

Respectfully submitted,

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Date: January 28, 2007

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